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厦门大学

博　士　学　位　论　文

日本囊对虾两种形态差异体形态性状、生长特性和耐热性比较研究

The morphology, growth characteristics and thermotolerance of the two morphologically similar varieties of *Marsupenaeus japonicus*

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缩略语中英文对照

缩写	英文	中文
aa	Amino acid	氨基酸
Amp	Ampicillin	氨卡青霉素
BLAST	Basic local alignment search tool	基本局域联配搜寻工具
bp	Base pair	碱基对
cDNA	Complementary DNA	互补脱氧核糖核酸
CTMax	Critical thermal maxima	临界高温
DEPC	Diethylpyrocarbonate	焦碳酸二乙脂
EDTA	Ethylene diamine teraacetic acid	乙二胺四乙酸
HSP	Heat shock protein	热休克蛋白
kDa	Kilodalton	千道尔顿
LB	Luria-Bertani medium	LB 培养基
mRNA	Messenger ribonucleic acid	信使 RNA
NCBI	National center for biotechnology information	美国国家生物信息中心
OR	Oxygen consumption rate	耗氧率
ORF	Open reading frame	开放阅读框
PCR	Polymerase chain reaction	聚合酶链式反应
PO	Phenoloxidase	酚氧化酶
RACE	Rapid amplfication of cDNA ends	快速扩增 cDNA 末端
ROS	Reactive oxygen species	活性氧
RT	Reverse transcription	反转录
SGR	Specific growth rate	特定生长率
TAE	Tris-acetic acid-EDTA buffer	Tris-乙酸 EDTA 缓冲液
THC	Total hemocyte counts	血细胞总数
UTR	Untranslated region	非翻译区
VIE	Visible implant elastomer	可视嵌入性荧光标记

中文摘要

日本囊对虾 *Marsupenaeus japonicus* (Bate, 1888)是我国沿海重要的经济养殖虾类，依据其头胸甲侧部斜纹全延伸与否可分为两种不同形态的差异体，二者地理分布不同并具较大遗传分化。以两类群体为材料，开展相关育种基础研究，具重要现实意义。本文首先研究比较了两种形态差异体群体生长特性，并通过多元分析确定影响其体质量的主要形态性状；其次，比较了二者的热耐受性，并初步探讨其耐高温机理；同时，克隆了日本囊对虾的 HSP60 基因并分析其热激时空表达，以期为更好开展日本囊对虾养殖生产和相关遗传育种提供实验依据和理论参考。主要研究结果如下：

1. 室内养殖环境下两种形态差异体群体生长特性研究

对室内养殖环境下两种形态差异体F_1群体不同性别对虾的体长和体质量进行跟踪测量，研究其生长特性及其规律。结果表明: 日本囊对虾体长和体质量呈幂函数关系，两群体雌、雄虾其体长、头胸甲长与体重的拟合参数b值皆近似为3，呈等速生长。拟合其von Bertallanffy 生长方程为:

形态差异体Ⅰ(♀): $L_t=15.932\times[1-e^{-0.0067(t-26.5055)}]$, $W_t=48.798\times[1-e^{-0.0067(t-26.5055)}]^{2.973}$;

形态差异体Ⅰ(♂): $L_t=13.642\times[1-e^{-0.0091(t-35.0053)}]$, $W_t=30.437\times[1-e^{-0.0091(t-35.0053)}]^{2.969}$;

形态差异体Ⅱ(♀): $L_t=13.865\times[1-e^{-0.0077(t-22.8107)}]$, $W_t=31.449\times[1-e^{-0.0077\ (t-22.8107)}]^{3.103}$;

形态差异体Ⅱ(♂): $L_t=12.094\times[1-e^{-0.0102(t-32.6776)}]$, $W_t=21.470\times[1-e^{-0.0102(t-32.6776)}]^{3.120}$。

进一步分析其极限生长年龄和生长拐点日龄得出，雌虾大于雄虾，形态差异体Ⅰ对虾大于形态差异体Ⅱ，表明前者最大体重高于后者而性成熟时间晚于后者。

2. 两种形态差异体群体形态性状对体质量的影响效果分析

随机选取室内相同养殖环境下两种形态差异体 F_1 群体对虾，分别测量 24 项形态指标和体质量(Y)性状。比较两群体各形态性状与体长的比例参数发现，二者在体形上存在一定差异。相关分析和通径分析结果表明，两群体对虾各形态性状与体质量的相关性均达到极显著($P<0.01$)水平；形态差异体Ⅰ群体对虾的体长(X_1)、第一腹节宽(X_{11})、头胸甲宽(X_{10})、第五腹节长(X_7)、第三腹节长(X_5)和第六腹节宽(X_{16})对体质量直接影响显著，形态差异体Ⅱ群体则以体长(X_1)、头胸甲长(X_2)、第五腹节宽(X_{15})和第一腹节宽(X_{11})对体质量直接影响显著；两群体决定系数分析结果与通径分析结果一致。经显著性分析，将偏回归系数显著的变量对体

质量建立多元回归方程，形态差异体Ⅰ: Y= -23.789+1.525X_1+5.183X_{10}+10.889X_{11}+5.671X_7－4.066X_5－4.054X_{16}，形态差异体Ⅱ: Y = -20.533+1.476X_1+4.759X_{11}+1.573X_2 + 6.864X_{15}，两个方程的回归关系均达到了极显著水平(P <0.01)。

3. 日本囊对虾 HSP60 基因 cDNA 克隆与热激表达

基于已知物种HSP60同源保守序列设计特异性引物，克隆获得日本囊对虾HSP60 cDNA全序列。序列全长2376bp，包括5’-UTR 80 bp，3’-UTR 556bp和ORF 1740bp。共编码579个氨基酸，推测其分子量为61.08 kD，等电点(pI)为5.49。氨基酸序列同源比对结果显示具较高的保守性，基于氨基酸序列构建的系统进化树亦与传统物种树基本吻合。荧光定量PCR研究表明，正常情况下日本囊对虾HSP60在血细胞、肝胰脏、心脏、鳃、胃、肠、肌肉和眼柄中均有表达，其表达程度在肝胰脏中最高，胃和眼柄中最低。热激后HSP60在鳃、心、肝胰脏、胃和肌肉组织中的表达水平显著(p <0.05)上调。鳃和肝胰腺中HSP60基因在热激后3h出现峰值，到12h时回落至正常水平。以上结果也证实了HSP60参与对虾热应激反应并在抵御热激过程中具有特殊作用，该基因热激后的表达情况或许可作为衡量评价对虾高温耐受性能的一项指标。

4. 两种形态差异体群体耐热性能及其机理研究

比较两种形态差异体群体高温环境下生长与成活，96h 亚致死温度以及两者在不同温度下的 CTMax 值，结果表明，形态差异体Ⅱ群体耐高温性能优于形态差异体Ⅰ群体，并认为 CTMax 是较好的对虾热耐受性能评价指标。机理探讨分析认为，形态差异体Ⅱ群体较低的耗氧率和窒息点可以更大限度的承受溶氧不足带来的危害，主要表现为相对较高的生长和成活率；另外，形态差异体Ⅱ群体鳃和肝胰腺组织中 HSP60 和 HSP90 基底水平较高表明对虾对可能来临的应激有更高的防御力，同时，热激后两组织中 HSP70 基因表达量显著低于形态差异体Ⅰ群体则表明其对高温应激的敏感性相对较弱，最终表现为更高的热耐受性。

关键词：日本囊对虾；形态差异体；生长特性；耐热性；热激蛋白

Abstract

Kuruma shrimp (*Marsupenaeus japonicus* Bate, 1888) is one of the most important species of shrimp culture along the coast of China. Previous study recognized two morphologically similar of *M. japonicus* and which were characterized by diagnostic color banding patterns on the carapace, moreover, these two varieties dad different geographical distribution and exsisted high genetic differentiation. In this study, comparisons of growth characteristics and morphological characters between the two morphologically similar varieties of *M. japonicus* were analysed, and the hermotolerance and its mechanism of the two morphologically similar varieties were investigated. Additionally, cDNA encoding a heat shock protein 60 (MjHSP60) gene in *M. japonicus* was cloned and the expression patterns and distribution profiles were investigated. The main results are as follows:

1. Growth characteristics of the two morphologically similar varieties of *Marsupenaeus japonicus* under indoor cultivation environment

The growth characteristics were studied on the basis of the successive data of body length and body weight of *Marsupenaeus japonicus* cultured in indoor cultivation environment. The results showed that the relationship between body length and body weight was described by the power function: Variety Ⅰ(♀): $W = 0.013\times L^{2.973}$, Variety Ⅰ(♂): $W = 0.013\times L^{2.960}$, Variety Ⅱ(♀): $W = 0.009\times L^{3.103}$, Variety Ⅱ(♂): $W = 0.009\times L^{3.120}$, where the value b were all close to 3, indicating an isometric growth. The von Bertallanffy equations were expressed as the follows:

Variety Ⅰ(♀): $L_t = 15.932 \times [1\text{-}e^{-0.0067(t\text{-}26.5055)}]$, $W_t = 48.798 \times [1\text{-}e^{-0.0067(t\text{-}26.5055)}]^{2.973}$;

Variety Ⅰ(♂): $L_t = 13.642 \times [1\text{-}e^{-0.0091(t\text{-}35.0053)}]$, $W_t = 30.437 \times [1\text{-}e^{-0.0091(t\text{-}35.0053)}]^{2.969}$;

Variety Ⅱ(♀): $L_t = 13.865 \times [1\text{-}e^{-0.0077(t\text{-}22.8107)}]$, $W_t = 31.449 \times [1\text{-}e^{-0.0077\ (t\text{-}22.8107)}]^{3.103}$;

Variety Ⅱ(♂): $L_t = 12.094 \times [1\text{-}e^{-0.0102(t\text{-}32.6776)}]$, $W_t = 21.470 \times [1\text{-}e^{-0.0102(t\text{-}32.6776)}]^{3.120}$.

Further analysis of their asymptotic value of body length and body weight and longevity, the results showed that the female was more than male and the Variety Ⅰ was more than Variety Ⅱ.

2. The effects of morphometric traits on body weight of two morphologically similar varieties of *Marsupenaeus japonicus*

Ninety individuals from two morphologically similar varieties of *Marsupenaeus japonicus* were randomly sampled respectively for measuring 24 morphometric traits including body length (X_1) , carapace length (X_2), the first ~ sixth abdominal segment length (X_3~X_8), telson length (X_9), carapace width (X_{10}), the first ~ sixth abdominal segment width (X_{11}~X_{16}), telson width (X_{17}), carapace height (X_{18}), the first ~ sixth abdominal segment height (X_{19}~X_{24}). The results for morphological characteristics showed that there is some difference in the body figure between the two varieties. By correlation and path analysis，the results showed that the correlation coefficients of each morphological trait to body weight (Y) were all at extremely significant level (P <0.01). The path coefficients of body length, the first abdominal segment width, carapace width, the fifth abdominal segment length, the third abdominal segment length and the sixth abdominal segment width of variety Ⅰ to body weight all reach significant level (P <0.05), but the direct effect of the last two traits to body weight is negative. The body length, carapace length, the fifth abdominal segment width and the first abdominal segment width of variety Ⅱ to body weight all reach significant level (P <0.05). The result of determination coefficients analysis was consistent with that of path in two varieties. The morphometric traits which reach level of significance(P <0.05) are used to establish the multiple regression equations，which were Y= - 23.789 + 1.525X_1 + 5.183X_{10} + 10.889X_{11} + 5.671X_7 — 4.066X_5 — 4.054X_{16} and Y = - 20.533 + 1.476X_1 + 4.759X_{11} + 1.573X_2 + 6.864X_{15}, respectively.

3. Cloning of HSP60 gene from the kuruma shrimp *Marsupenaeus japonicus* and its tissue expression before and after heat shock

As a major member of heat shock protein families, HSP60 play an important role in the life activities of cells. In the present study, cDNA encoding a heat shock protein 60 (MjHSP60) gene in *Marsupenaeus japonicus* was cloned based on the homologous conserved sequence and rapid amplification of cDNA end (RACE) methods. The full length of the MjHSP60 cDNA was found to be 2376bp, with 5'-UTR (untranslated Regions) of 80bp, 3'-UTR of 556bp and a 1740bp open reading frame. The translated

amino acid sequence consisted of 579 residues with a calculated molecular mass of 61.08 kD and an theoretical isoelectronic point (pI) of 5.49. Comparison of the deduced amino acid sequence showed that it has high identity (68~93%) with HSP60 from other eukaryotes. Quantitative real-time PCR analysis were carried out to investigate the expression patterns and distribution profiles of MjHSP60 before and after heat shock. MjHSP60 mRNA was highly expressed in gills and hepatopancreas and almost all tissues examined, including haemocytes, muscle, stomach and eyestalk, but it was less strongly expressed in the intestine. The expression analysis revealed that MjHSP60 was significantly up-regulated in the gills, hepatopancreas, heart and haemocytes after heat shock. The expression level of HSP60 gene in gills and hepatopancreas reached a peak at 3h and 12 hand fall back to normal level after heat shock.

4. Thermotolerance and its mechanism of the two morphologically similar varieties of *Marsupenaeus japonicus*

Comparisons of the growth and survival under high temperature, 96h sub-lethal temperature as well as the CTMax value under different temperatures between the two morphologically similar varieties of *Marsupenaeus japonicus* were analysed, the results showed that the thermotolerance of variety Ⅱ was better than variety Ⅰ, and found that CTM can be a better evaluation index for thermotolerance of prawn. The results of mechanism analysis showed that: (1) the lower oxygen consumption rate and asphyxiation point could protect variety Ⅱ from the hazards of oxygen deficiency, showed a relatively high growth and survival rate; (2) the higher basal level of HSP60 and HSP90 in gills and hepatopancreas of variety Ⅱ could provide them higher resistance on heat shock; (3) the expression of HSP70 and HSP90 gene after heat shock in gills and hepatopancreas of variety Ⅱ were significantly(P <0.05) lower than that in variety Ⅰ, indicated that the susceptibility to heat stress of variety Ⅱ was weaker than variety Ⅰ.

Key Words: *Marsupenaeus japonicus*; morphologically similar variety; growth characteristics; thermotolerance; heat shock protein